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Toxicological Assessment of Hexachlorobiphenyl Isomers and 2,3,7,8-Tetrachlorodibenzofuran in Chicks

II. Effects on Drug Metabolism and Porphyrin Accumulation[1]

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Toxicological Assessment of Hexachlorobiphenyl Isomers and 2,3,7,8-Tetrachlorodibenzofuran in Chicks. II. Effects on Drug Metabolism and Porphyrin Accumulation. Goldstein, Joyce A., McKinney, James D., Lucier, George W., Hickman, Patricia, Bergman, Hinda and Moore, John A. (1976). *Toxicol. Appl. Pharmacol.* 36, 81–92. Pure hexachlorobiphenyl (HCB) isomers induce a number of changes in parameters of drug metabolism in the chick including changes in cytochrome P-450, liver weight, and *p*-nitrophenol glucuronyl transferase, but not in testosterone glucuronyl transferase activity. The most active inducers of drug metabolism were 2,3,4,2′,3′,4′-HCB and 2,4,6,2′,4′,6′-HCB, while 2,4,5,2′,4′,5′-HCB produced intermediate effects and 2,3,6,2′,3′,6′-HCB was a poor inducer. All HCBs caused uroporphyrin accumulation and increased δ-aminolevulinic acid (ALA) synthetase activity, but only 3,4,5,3′,4′,5′-HCB, 2,3,4,2′,3′,4′-HCB, and 2,4,5,2′,4′,5′-HCB produced gross accumulation of hepatic porphyrins. Tissue HCB concentrations correlated well with hepatic effects. 2,3,7,8-Tetrachlorodibenzofuran (TCDF), a contaminant of commercial polychlorinated biphenyl (PCB) mixtures, had no effects on hepatic ALA synthetase activity, porphyrin accumulation, or glucuronyl transferase. TCDF did produce a slight increase in cytochrome P-450, but the increase was smaller than that produced by HCBs. Also, the cytochrome induced by TCDF appeared qualitatively different from the cytochrome induced by HCBs, differing by a shift in the peak of the CO-difference spectrum from 450 to 449 nm and by the ratio of absorbance at 455 to 430 nm of the ethyl isocyanide difference spectrum.

Polychlorinated biphenyls (PCBs) are industrial chemicals which are widely distributed in the environment. They have been found in tissues of wildlife and humans (Risebrough *et al.*, 1968; Biros *et al.*, 1970). Commercial mixtures of PCBs have been shown to increase liver weight, cytochrome P-450 and activity of a number of drug-metabolizing

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enzymes including glucuronyl transferase, and to produce experimental hepatic porphyria (Litterst *et al.*, 1972; Vos *et al.*, 1971; Goldstein *et al.*, 1974a).

In general, the stimulatory effect of PCB mixtures on hepatic enzymes appears to be related to the chlorine content of the biphenyl, since potency increases with increasing percent of chlorination (Litterst *et al.*, 1972). However, two PCB mixtures with identical chlorine content by weight but different isomer composition (Aroclor 1242 and Aroclor 1016) are quite different in their effects on microsomal enzymes (Goldstein *et al.*, 1975). Little work has been done thus far comparing effects of isomerically pure chlorobiphenyl compounds. Johnstone *et al.* (1974) recently compared a number of chlorinated biphenyls in the rat at a single-dose level and found 4,4-di-, 2,4,2′,4′-tetra-, 2,4,5,2′,4′,5′-, 2,3,5,2′,3′,5′-, and 2,4,6,2′,4′,6′-hexa-, 2,3,4,5,2′,3′,4′,5′-octa-, and 2,3,4,5,6,2′,3′,4′,5′,6′-decachlorobiphenyls to be extremely active inducers of a number of drug-metabolizing enzymes. However, 2,2′- and 2,4′-dichlorobiphenyl were considerably less active, as was 2,5,2′,5′-tetrachlorobiphenyl, indicating that the position of chlorines on the biphenyl is important for activity. Fujita *et al.* (1971) found 2,4,5,3′,4′-penta and 3,4,5,3′,4′,5′-hexachlorobiphenyls to be more active inducers of hepatic drug-metabolizing enzymes than 4,4′-di- and 3,4,2′,4′-tetrachlorobiphenyls, suggesting that the number of chlorines per biphenyl may be important.

Complicating the situation even more, several commercial preparations of PCBs contain traces of the highly toxic chlorinated 2,3,7,8-tetrachlorodibenzofuran (TCDF) (Vos *et al.*, 1970). The chlorinated dibenzo-*p*-dioxins and dibenzofurans have been reported to increase both drug-metabolizing enzymes and δ-aminolevulinic acid (ALA) synthetase, the rate-limiting enzyme in heme and porphyrin synthesis (Lucier *et al.*, 1973; Poland and Glover, 1973), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCCD) has been found to be porphyrogenic to mammals (Goldstein *et al.*, 1973). Little work has been done on the mammalian effects of TCDF.

The present study was undertaken to compare effects of several hexachlorobiphenyl isomers on a number of parameters of hepatic function in the chick, including induction of hepatic drug-metabolizing enzymes and precipitation of hepatic porphyria. These effects were compared with those of TCDF.

METHODS

Chemicals. 2,4,5,2′,4′,5′-Hexachlorobiphenyl (HCB) (>99% pure), 2,3,6,2′,3′,6′-HCB (>99% pure), and 3,4,5,3′,4′,5′-HCB (66% pure) were synthesized at NIEHS (McKinney *et al.*, 1976). The impurity in 3,4,5,3′,4′,5′-HCB was 2,3,4,5,3′,4′,5′-heptachlorobiphenyl. A second batch of 3,4,5,3′,4′,5′-HCB (99% pure) was later synthesized by McKinney *et al.* (1976) by an alternate method. 2,4,6,2′,4′,6′-HCB (>99% pure) and 2,3,4,2′,3′,4′-HCB (>99% pure) were purchased from Analabs, Inc. TCDF (88% pure) was synthesized by Dr. A. S. Kende, University of Rochester, Rochester, New York. The contaminant was 1,2,3,7,8-pentachlorodibenzofuran.

Animals. One-day-old White Leghorn cockerels were obtained from Central Carolina Farmers, Durham, North Carolina. In the first experiment, the chicks were fed a diet formulated for chick edema bioassay from Nutritional Biochemicals, Cleveland, Ohio, containing 0 or 400 ppm of each of the hexachlorobiphenyls listed above or dosed

orally with 1 or 5 μg/kg/day of TCDF in 0.1 ml of acetone:corn oil (1:7). The 3,4,5,3′,4′,5′-isomer used was from the first synthesis (66% pure). Another group of chicks received 0.1 ml of acetone:corn oil (1:7). The initial number of birds per group varied from 6 to 10. Cage temperature was maintained at 30°C for 2 weeks, then lowered to room temperature (25°C) at the beginning of week 3. After 3 weeks, surviving chicks were killed by carbon dioxide anesthesia. Liver enzymes were determined on four to five animals per group, and liver porphyrins on all animals. In a second experiment, chicks were fed 0, 100, and 300 ppm of both 66 and 99% pure 3,4,5,3′,4′,5′-HCB (10 chicks per group).

Liver. A portion of the liver was excised, homogenized in ice-cold 0.2 M HEPES buffer, pH 7.4, microsomes prepared (Goldstein *et al.*, 1974a), and the latter resuspended in 0.2 M HEPES buffer for assay of glucuronyl transferase, or in 1.15% KCl containing 0.1 M K_2HPO_4–KH_2PO_4 buffer (pH 7.4) and 25% glycerol for cytochrome P-450 analysis. The resuspended pellet was stored overnight at 0°C before determination of cytochrome P-450 and ethyl isocyanide ratios.

Enzyme assays. ALA synthetase activity was assayed in whole homogenates of a second portion of liver (Goldstein *et al.*, 1974a). *p*-Nitrophenol glucuronyl transferase (Lucier *et al.*, 1971) and testosterone glucuronyl transferase activities (Lucier, 1974) were determined in Triton X-100 treated microsomes.

Microsomal cytochromes and protein. Microsomal protein was measured by the method of Lowry *et al.* (1951) using crystalline albumin as the standard. Microsomal cytochrome P-450 (Omura and Sato, 1964) and ethyl isocyanide ratios (Sladek and Mannering, 1966) were measured using a DW-2 spectrophotometer.

Porphyrins. Total liver porphyrins were determined by extraction into 0.9 M perchloric 1-1 ethanol (1:1, v/v) and measured fluorometrically with uroporphyrin as a standard (Abritti and De Matteis, 1971–1972). Liver porphyrins of some samples were extracted from 0.5 to 1 g of liver, separated by thin-layer chromatography (Doss, 1967), and quantitated as previously described (Goldstein *et al.*, 1974a).

Tissue residue analysis. Subcutaneous adipose tissue and liver samples were pooled within each treatment group, extracted, and hexachlorobiphenyl residue concentrations determined by gas–liquid chromatography with a flame ionization detector (McKinney *et al.*, 1976).

RESULTS

Chicks fed 400 ppm of 66%, 3,4,5,3′,4′,5′-HCB or dosed orally with 5 μg/kg of TCDF died before sacrifice, preventing determination of their effects on liver enzymes. Results in Fig. 1 show that all other hexachlorobiphenyl isomers tested increased cytochrome P-450 content of chick livers (4- to 14-fold). 2,3,4,2′,3′,4′-HCB produced significantly greater and 2,3,6,2′,3′,6′-HCB significantly less effects than other isomers. TCDF (1 μg/kg) increased cytochrome P-450 significantly over corn oil-treated controls (threefold), but less than all HCB isomers other than 2,3,6,2′,3′,6′-HCB. The peak of the CO-difference spectrum was shifted from 450 nm for controls to 449 nm by TCDF, but remained at 450 nm in the livers of PCB-treated animals.

Liver weight was increased significantly by all PCBs except 2,3,6,2′,3′,6′-HCB (Fig. 1). The pattern was slightly different than for cytochrome P-450, in that 2,3,4,2′,3′,4′-HCB no longer produced the greatest effects. The largest increase was produced by

2,4,6,2′,4′,6′-HCB ,which was significantly greater than that produced by 2,4,5,2′,4′,5′-HCB or 2,3,6,2′,3′,6′-HCB. The second largest increase (2,3,4,2′,3′,4′-HCB) was significantly larger than that produced by 2,3,6,2′,3′,6′-HCB. TCDF did not significantly increase liver weight.

The sequence of effects on microsomal protein were similar to those on cytochrome P-450, 2,3,4,2′,3′,4′-HCB producing the largest increase and 2,3,6,2′,3′,6′-HCB the least (Fig. 1). Only 2,3,4,2′,3′,4′-HCB, 2,4,6,2′,4′,6′-HCB, and TCDF produced

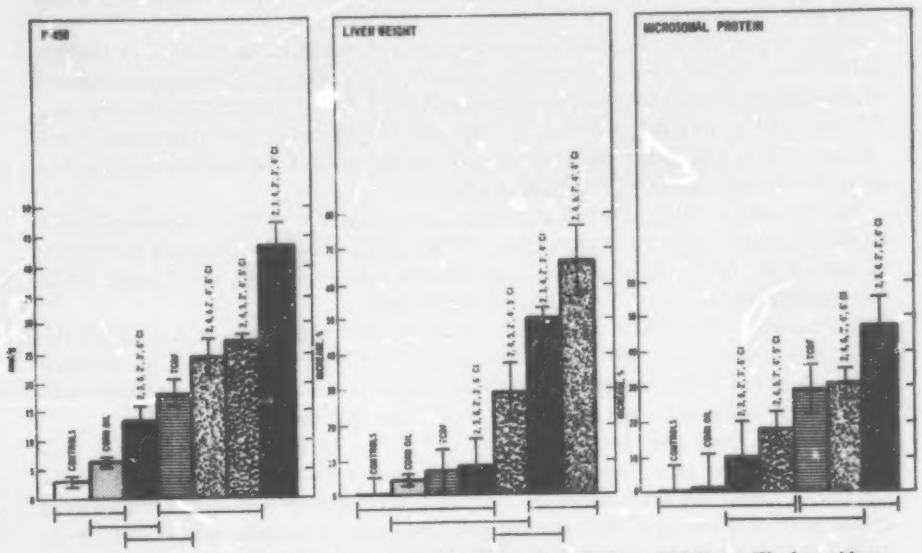

FIG. 1. Effects of purified hexachlorobiphenyl isomers and TCDF on chick livers. The hexachlorobiphenyl isomers indicated above were fed to day-old chicks at a dietary concentration of 400 ppm for 21 days. TCDF was given orally at a dose of 1 μg/kg daily for 21 days in acetone:corn oil (1:7). Controls for this group were dosed daily with acetone:corn oil (1:7). Vertical bars represent the means ± SE. Control values for liver weight were 7.3 ± 0.4 g and for liver microsomal protein were 23.9 ± 1.9 mg/g. Results were analyzed by Duncan's Multiple-Range test (Steel and Torrie, 1960), and the heavy lines below the bar graph indicate groups which do not significantly differ from each other ($p < 0.05$). Groups not underlined by the same line are significantly different.

significant increases. The effects of 2,3,4,2′,3′,4′-HCB were significantly greater than those of 2,3,6,2′,3′,6′-HCB or 2,4,5,2′,4′,5′-HCB.

All PCBs increased *p*-nitrophenol glucuronyl transferase activity (14- to 23-fold) (Fig. 2). Again the smallest increase was seen with 2,3,6,2′,3′,6′-HCB and the largest increase with 2,3,4,2′,3′,4′-HCB. Unlike *p*-nitrophenol glucuronyl transferase activity, testosterone glucuronidation was not affected by any of the compounds tested.

Table 1 shows the effects of hexachlorobiphenyl isomers and TCDF on the ethyl isocyanide ratios of chick liver microsomes. None of the HCB isomers had any effect on the ethyl isocyanide ratios, but TCDF did increase the ratio slightly.

As shown in Table 2, all PCBs tested produced similar increases in ALA synthetase activity (five- to six-fold), unlike the differential effects on liver weight and cytochrome

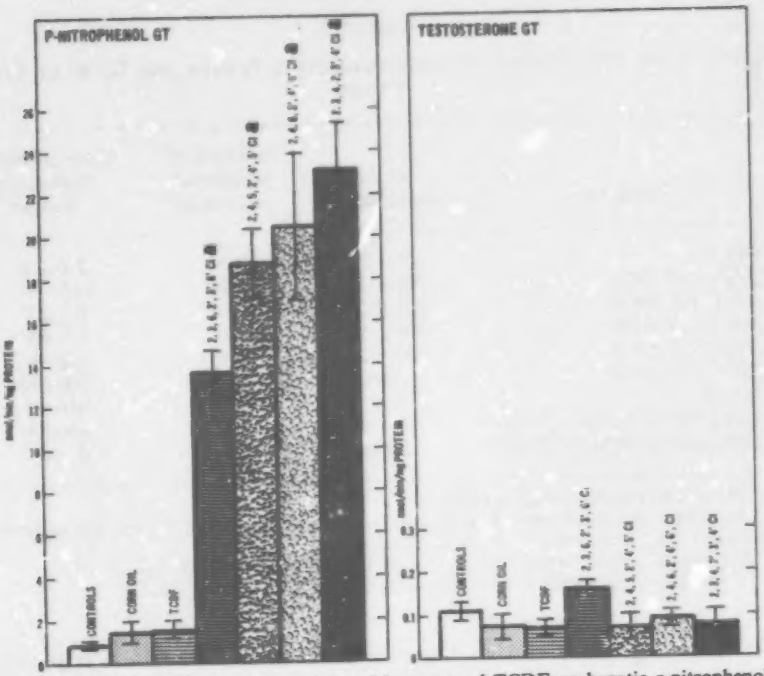

FIG. 2. Effects of purified hexachlorobiphenyl isomers and TCDF on hepatic *p*-nitrophenol and testosterone glucuronyl transferase activities. Day-old male chicks were fed 400 ppm of the above hexachlorobiphenyls or dosed orally with 1 μg/kg of TCDF in acetone:corn oil (1:7) for 21 days. Vertical bars represent the mean ± SE. [a] Significantly different from controls; $p < 0.05$ (Student's *t* test).

TABLE 1

EFFECTS OF HEXACHLOROBIPHENYL ISOMERS AND TCDF ON ETHYL ISOCYANIDE DIFFERENCE SPECTRA IN THE CHICK[a]

Treatment	455/430 ratio
Controls	0.42 ± 0.01
2,4,5,2′,4.′5′,-HCB	0.40 ± 0.04
2,3,6,2′,3′,6′-HCB	0.40 ± 0.10
2,4,6,2′,4′,6′,-HCB	0.37 ± 0.01
2,3,4,2′,3′,4′-HCB	0.43 ± 0.01
Corn oil	0.51 ± 0.07
1 μg/kg of TCDF	0.74 ± 0.06[b]

[a] Treatment is described in the legend of Fig. 1. Values represent the means ± SE.

[b] Significantly different from controls. $p < 0.05$ (Duncan's Multiple-Range test).

TABLE 2

EFFECT OF 400 PPM PURIFIED HEXACHLOROBIPHENYL ISOMERS AND TCDF ON CHICK LIVERS[a]

Treatment	ALAS (nmol/g/hr)	Incidence of porphyria[b] (>10 μg/g)	Liver porphyrin levels (μg/g) (range)
Control	55 ± 2	0/10	0.4–1.4
2,3,6,2′,3′,6′-HCB	261 ± 15[c]	0/10[d]	0.5–1.4
2,4,6,2′,4′,6′-HCB	303 ± 15[c]	0/10[d]	0.7–2.9
2,4,5,2′,4′,5′-HCB	309 ± 13[c]	3/9[d]	1.5–82
2,3,4,2′,3′,4′-HCB	250 ± 28[c]	2/8[d]	1.2–81
3,4,5,3′,4′,5′-HCB	Dead	1/7[d]	2.4–5000
Corn oil	74 ± 2	0/3	0.4–1.2
1 μg/kg/day of TCDF × 21 days	63 ± 20	0/5	0.4–2.9
5 μg/kg/day of TCDF × 21 days	Dead	0/5	0.3–0.7

[a] Treatment described in legend of Fig. 1.
[b] Proportion of animals whose liver porphyrin concentrations exceed 10 μg/g. The upper 99% tolerance levels for controls at $p < 0.01$ is 2.3 μg/g.
[c] Significantly greater than controls, $p < 0.05$ (Duncan's Multiple-Range test).
[d] Uroporphyrin detectable in all samples after thin-layer chromatography.

P-450. In contrast, TCDF produced no effect. Liver porphyrins were determined in all groups, including animals which died or were killed or moribund before 21 days. Table 2 shows the incidence of porphyria, defined as a porphyrin concentration greater than 10 μg/g of liver (control = 0.8 ± 0.1 μg/g), and the range of each group. The upper tolerance limit for 99% of control samples at $p < 0.01$ is 2.3 μg/g. Many of the animals in all groups were within the tolerance limits for control samples. However, 3/9 of the samples fed 400 ppm 2,4,5,2′,4′,5′-HCB, 2/8 of the samples fed 2,3,4,2′,3′,4′-HCB, and 1/7 of the samples fed 3,4,5,3′,4′,5′-HCB (died or were killed moribund before 21 days) were grossly porphyric. Although no animals fed 2,3,6,2′,3′,6′-HCB were porphyric, neither were any animals fed 2,4,6,2′,4′,6′-HCB porphyric. Extraction, purification, and thin-layer chromatography were performed on two to three liver samples from each treatment group. Only traces of protoporphyrin and coproporphyrin were found in control livers, but traces of 8- and 7-carboxyporphyrins were seen in all PCB-treated livers analyzed, even from those animals fed 2,3,6,2′,3′,6′-HCB and 2,4,6,2′,4′,6′-HCB. Only 8- and 7-carboxyporphyrins could be detected in porphyric livers. Porphyrin also accumulated in the crop and bones. The porphyrin in the crop was 4-, 7-, and 8-carboxyporphyrins (31, 20, and 49%, respectively), while the porphyrin in the bones was entirely 7- and 8-carboxyporphyrins (27 and 73%). None of the animals fed 1 or 5 μg/kg of TCDF showed any accumulation of porphyrins, even after extraction and thin-layer chromatography.

In the second experiment, chicks fed 0, 100, or 300 ppm of 66% pure (crude) versus 99% pure (pure) 3,4,5,3′,4′,5′-HCB died before 21 days; therefore, effects on liver enzymes were not determined. Liver porphyrins were elevated by both pure and crude 3,4,5,3′,4′,5′-HCB at both dietary levels (Table 3).

TABLE 3

EFFECT OF 3,4,5,3′,4′,5′-HEXACHLOROBIPHENYL ON HEPATIC PORPHYKINS[a]

Treatment	Incidence of porphyria[b]	Liver porphyrins[c] (μg/g)
Control	0/10	0.66 ± 0.07
100 ppm 3,4,5,3′,4′,5′-HCB(66% pure)	5/8	16.7 ± 11.2[d]
300 ppm 3,4,5,3′,4′,5′-HCB(66% pure)	7/7	200 ± 53[d]
100 ppm 3,4,5,3′,4′,5′-HCB(99% pure)	8/8	68 ± 42[d]
300 ppm 3,4,5,3′,4′,5′-HCB(99% pure)	7/7	267 ± 127[d]

[a] Two synthetic batches of 3,4,5,3′,4′,5′-HCB (66% and 99% pure) were fed to chicks at the dietary concentrations indicated for 21 days. All chicks fed 100 or 300 ppm of either batch of the HCB died before 21 days, and liver samples were taken at autopsy.

[b] Proportion of animals whose liver porphyrins exceed the upper 99% tolerance limit for controls at $p < 0.01$ (1.75 μg/g).

[c] Values represent means ± SE.

[d] Significantly greater than controls, $p < 0.05$ (Student's t test).

Toxicity expressed as death (groups fed 400 ppm 3,4,5,3′,4′,5′-HCB or dosed orally with 5 μg/kg/day of TCDF) or body weight loss (Fig. 3) appears unrelated to effects on the liver. 2,4,5,2′,4′,5′-HCB and 2,3,4,2′,3′,4′-HCB caused significant weight loss, while 2,4,6,2′,4′,6′-HCB produced no weight loss.

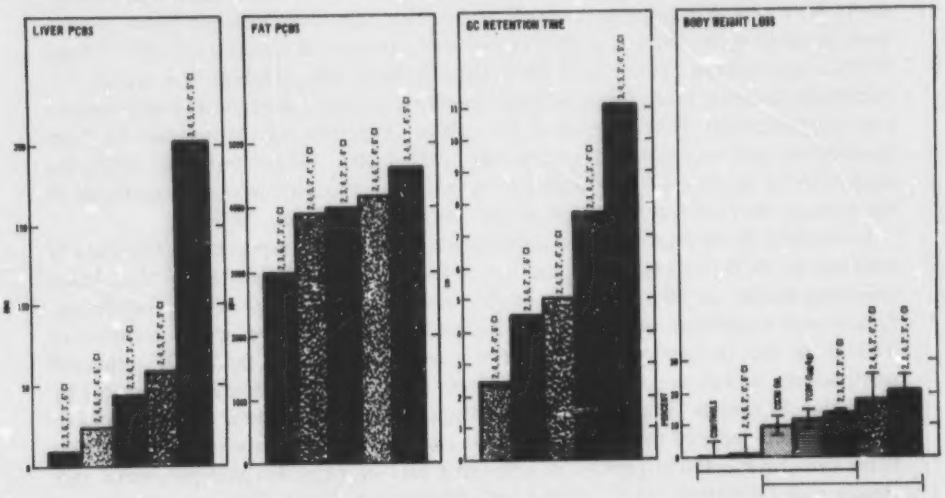

FIG. 3. Liver and fat concentration of hexachlorobiphenyls and body weight loss of chicks fed purified hexachlorobiphenyl isomers. Chicks were treated as described in Fig. 1. Values for liver and fat concentrations of PCBs are the results of duplicate analyses of pooled liver samples. Body weight loss is reported as percentage of control of body weight (203 ± 10 g). Values represent means ± SE, and heavy lines beneath bars indicate groups which do not differ significantly, $p < 0.05$ (Duncan's Multiple-Range test).

The PCB concentration of fat paralleled effects on liver weight (Fig. 3). 3,4,5,3′,4′,5′-HCB which was lethal at 400 ppm in the diet, produced the highest liver and fat PCB content. The 2,3,6,2′,3′,6′-isomer accumulated the least and produced the least liver effects. GC retention times (Fig. 3) correlate reasonably well with HCB content of fat, except for the relatively low retention time and high fat levels and biological activity of 2,4,6,2′,4′,6′-HCB.

DISCUSSION

Our results show that pure hexachlorobiphenyls alter a number of parameters of hepatic function in the chick, including cytochrome P-450, microsomal protein, liver weight, and glucuronyl transferase activity. There were distinct differences in the induction produced by the hexachlorobiphenyl isomers tested: 2,3,4,2′,3′,4′-HCB and 2,4,6,2′,4′,6′-HCB were the most active inducers, 2,4,5,2′,4′,5′-HCB produced intermediate effects, and 2,3,6,2′,3′,6′-HCB was a relatively poor inducer of the above biochemical parameters. These results show that even within the highly chlorinated hexachlorobiphenyls, position of the chlorines is important for activity. The relative inactivity of the 2,3,6,2′,3′,6′-hexa- isomer indicated that occupation of the *p*-position may be important. The work of Johnstone *et al.* (1974) also indicated that occupation of the *para*-positions may be a very important factor governing ability to induce liver enzymes, especially with PCB isomers with low chlorine content. Differences among the more potent hexa- isomers were not obvious, probably because the dose utilized produced a maximum increase. An additional lower dose of the hexachlorobiphenyls might have revealed differences in this group. The differences between hexa- isomers were possibly more obvious in the present study because the increase in cytochrome P-450 is much greater in the chick (14×) than in the rat (4×) (Goldstein *et al.*, 1974a). Although glucuronyl transferase activity in microsomes of control chicks was extremely low, approximately 1/30 of those of rat, rabbit, or guinea pig microsomes for both testosterone and *p*-nitrophenol (Lucier *et al.*, 1971; Lucier, 1974; Hook *et al.*, 1975), the magnitude of the increase produced by the hexachlorobiphenyl isomers was similar to the increase produced by PCB mixtures in rats (Goldstein *et al.*, 1975).

In contrast to the purified hexachlorobiphenyls, TCDF, a suspected contaminant of commercial PCB mixtures, produced less induction of cytochrome P-450 than hexachlorobiphenyls, no effect on liver weight, and no effect on glucuronyl transferase. Glucuronyl transferase activity was stimulated markedly by the related compound, TCDD, in rats (Lucier *et al.*, 1973). The cytochrome induced by TCDF appeared qualitatively, as well as quantitatively, different from the cytochrome P-450 induced by hexachlorobiphenyls. Similarly, administration of certain polycyclic hydrocarbons or TCDD to rats induced the formation of cytochrome P-448, a cytochrome that differs from cytochrome P-450 present in untreated rats or phenobarbital-pretreated rats (Greig and DeMatteis, 1973; Sladek and Mannering, 1966). The new cytochrome differed from cytochrome P-450 by a 2-nm shift in the peak of the CO-difference spectrum, increase in the ratio of the 455 to 430 nm peaks of the ethyl isocyanide difference spectrum, and the substrate specificity. Administration of PCBs, on the other hand, appeared to increase formation of both cytochromes P-450 and P-448 in rats: the peak of the CO-difference spectrum was shifted to 448 nm, but the ratio of the

455 nm to the 430 nm peaks of the ethyl isocyanide difference spectrum was changed to a value intermediate between those found after treatment with polycyclic hydrocarbons and phenobarbital, and there was increased metabolism of both substrates metabolized primarily by cytochrome P-450 and cytochrome P-448 (Alvares *et al.*, 1975). In the present study, only TCDF shifted the peak of the CO-difference spectrum and altered the ratio of the 455/430 nm ethyl isocyanide peaks. The apparent formation of cytochrome P-450 rather than cytochrome P-448 after administration of PCB isomers was unexpected, and may reflect either a species difference or differences between isomers. Alternately, induction of cytochrome P-448 by PCB mixtures may be a result of contamination by TCDF.

We previously reported that PCB mixtures produced hepatic porphyria, accumulation of uroporphyrins, and increased activity of ALA synthetase in the rat (Goldstein *et al.*, 1974a). Only three of the hexachlorobiphenyls tested in the present study produced an incidence of porphyria: 2,4,5,2′,4′,5′-HCB, 2,3,4,2′,3′,4′-HCB, and 3,4,5,3′,4′,5′-HCB. Interestingly, these isomers were also the most toxic, as measured by death or weight loss. It is notable that all three of these isomers had both *m*- and *p*-positions occupied by chlorines, while 2,3,6,2′,3′,6′-HCB and 2,4,6,2′,4′,6′-HCB with *p*- or *m*-positions, respectively, unoccupied were inactive. Alternatively, the nonplanarity provided by the 4 *ortho* chlorines may have decreased the porphyrogenic activity of these two compounds. However, all hexachlorobiphenyl isomers tested increased ALA synthetase and produced a slight accumulation of uroporphyrins. The time course of this disease has not been studied in chickens, but there is a lag of several weeks before porphyria and increased activity of ALA synthetase can be demonstrated in rats fed PCBs (Goldstein *et al.*, 1974a). The induction of ALA synthetase and slight increase in uroporphyrin accumulation in all chicks suggests that all hexachlorobiphenyls tested were porphyrogenic; however, there is probably a lag period in the onset of the disease, and some hexachlorobiphenyls appear more porphyrogenic than others. Reports that certain tetrachlorobiphenyl isomers did not increase ALA synthetase when administered acutely to rats (Chen *et al.*, 1973) are of doubtful significance, since similar doses of PCB mixtures did not increase ALA synthetase activity when given acutely to rats, but increased activity when fed chronically (Goldstein *et al.*, 1974a).

The presence of small amounts of TCDF in some commercial PCB mixtures (Vos *et al.*, 1970) has led many authors to speculate that the porphyrogenic effects of PCBs could be produced by contamination with TCDF. The related compound TCDD was porphyrogenic to mice at doses of 25 μg/kg/week × 4 weeks (Goldstein *et al.*, 1973), and both TCDD and TCDF have been shown to induce ALA synthetase and aryl carbon hydroxylase in the chick embryo at approx 5×10^{-11} mol/egg (Poland and Glover, 1973). The present study shows that TCDF did not produce porphyria in chicks, even at lethal doses, or increase ALA synthetase. We have also shown that TCDF does not produce accumulation of porphyrins in livers of mice at doses as high as 4 mg/kg (unpublished observations; Goldstein *et al.*, 1974b). These results suggest strongly that TCDF plays no significant role in the production of porphyria by PCB mixtures. Similarly, the relatively lesser effects of TCDF than hexachlorobiphenyls on liver enzymes make it extremely unlikely that any significant portion of the effects of PCB mixtures could be due to contamination with trace amounts of TCDF. Possibly, differences in absorption, distribution, or metabolism of TCDF versus TCDD may

explain the relative inactivity of TCDF as an inducer of porphyria and drug-metabolizing enzymes in mammals and adult chickens, whereas both compounds are very potent in the chick embryo.

Tissue PCB concentrations can partially explain the differences in hexachlorobiphenyl isomers as hepatic inducers. The 3,4,5,3′,4′,5′-isomer accumulated the most in fat and liver, caused porphyria and was lethal. The 2,3,6,2′,3′,6′-isomer accumulated the least in both fat and liver, and produced the smallest increase in all parameters. However, some differences in hepatic effects (2,3,4,2′,3′,4′, > 2,4,5,2′,4′,5′) cannot be explained by liver concentrations. Concentrations of HCBs in fat correlated well with liver effects. This may suggest that lipophilicity is an important factor in hepatic potency. GC retention indices also correlated reasonably well with liver effects with the exception of 2,4,6,2′,4′,6′-HCB. This isomer and 2,3,6,2′,3′,6′-HCB both have four *ortho* chlorines which would tend to hinder rotation and make the compound nonplanar. The 2,4,6,2′,4′,6′-isomer is highly symmetrical, however, adding to its polarity. GC retention times may reflect lipophilicity with the exception of biphenyls which have four *ortho* positions occupied by chlorine. Alternatively, 2,3,6,2′,3′,6′-HCB could exist as a racemic mixture because of steric hindrance provided by the *ortho* chlorines. It is possible that one form is relatively inactive, resulting in lower activity for the mixture. Significantly, Bush *et al.* (1974) and Baker *et al.* (1974) found that tissue persistence of individual isomers in chickens fed Aroclor 1254 correlated with *para*-substitution and mixed-function oxidase activity especially for the penta- and hexa- isomer.

Tissue PCB concentrations may be an important factor in biological effectiveness. It is well known that some isomers, particularly the lower chlorinated homologs, are preferentially metabolized and excreted over highly chlorinated homologs (Weigel and Smith, 1974). On the other hand, studies with Aroclor mixtures with comparable chlorine content but differing isomer composition (i.e., Aroclors 1242 and 1016) demonstrated marked differences in biological potency despite comparable tissue concentrations (Goldstein *et al.*, 1975). The question remains as to the extent to which differences in absorption, metabolism, and distribution determine biological activity of polychlorinated biphenyl isomers. Future studies should direct further attention to the importance of the position and number of chlorines on the biphenyl nucleus in determining biological activity, and the relationship of these parameters to metabolism and tissue distribution.

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